

# Wheat-rye translocations

## Detection of chromosome breakpoints by in situ hybridization with a biotin-labeled DNA probe

**ABSTRACT:** In situ hybridization with a biotin-labeled DNA probe was used to detect wheat-rye translocations. The probe containing a 120-bp repetitive DNA sequence from rye, hybridized to the entire length of all rye chromosomes, but only to a few sites in 14 wheat chromosomes. The overall distribution of this DNA probe in the rye chromosomes has not been detected previously with the use of radioactively labeled probes. As a result of the formation of a brown precipitate over sites of hybridization in this technique, the rye chromosomes were entirely brown in color, whereas the wheat chromosomes appeared blue. The distinguishable appearance of the wheat and rye chromosomes resulted in an efficient and sensitive method of detecting translocations.

N. L. V. Lapitan  
R. G. Sears  
A. L. Rayburn  
B. S. Gill

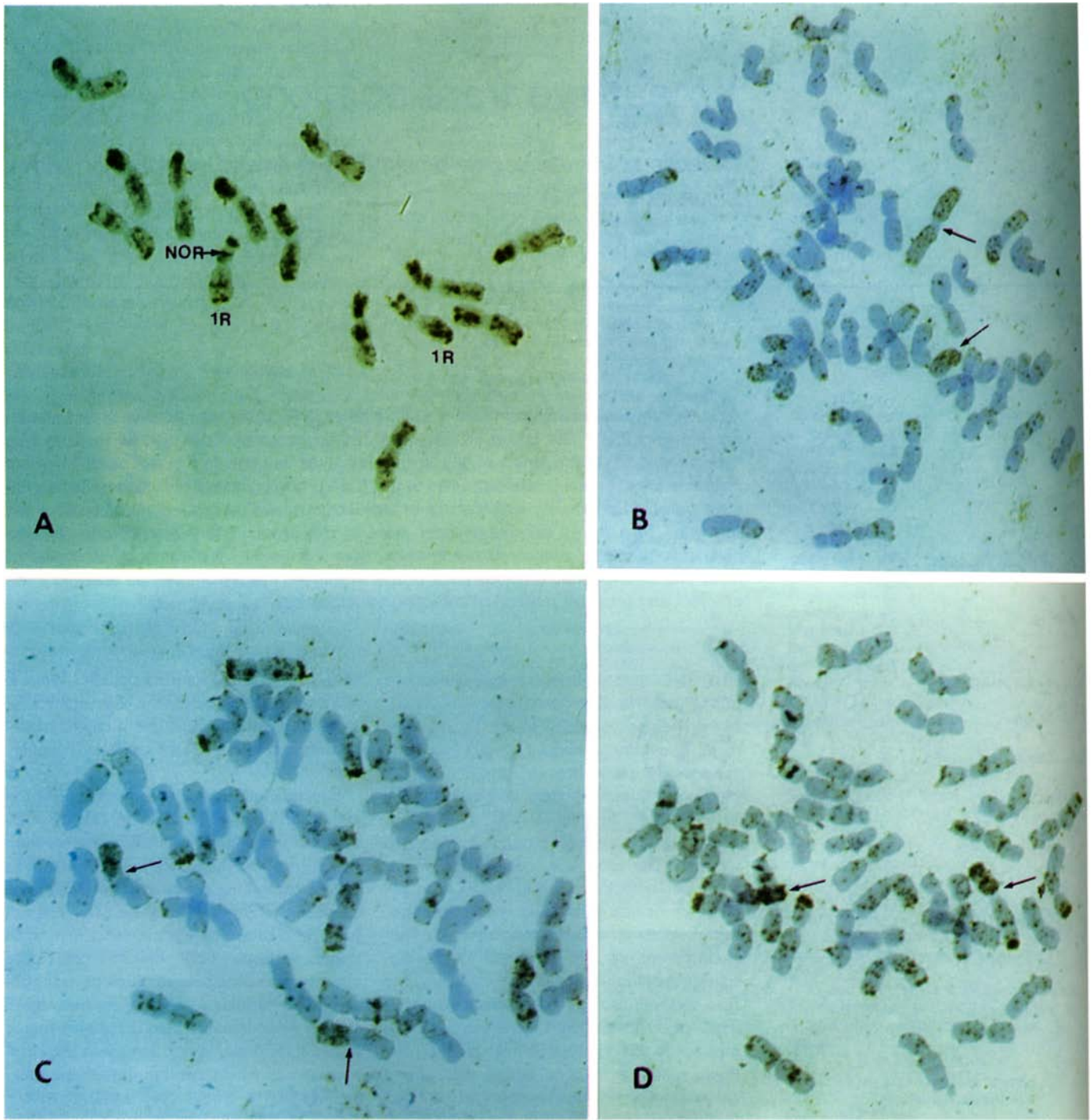
THE INCORPORATION of rye chromatin into wheat, via chromosome translocations, has proven to be useful in wheat improvement. By means of wheat-rye translocations, rye genes for resistance to pathogens, such as powdery mildew<sup>17,23</sup>, stem and leaf rust<sup>4,22</sup>, insects, including wheat curl mite<sup>15</sup>, and greenbug<sup>20</sup>, have been transferred into wheat<sup>21</sup>. The most commonly used procedures for detecting such translocations include 1) meiotic pairing analysis<sup>19</sup>, 2) use of morphogenetic, disease resistance, or biochemical markers<sup>8,9,17,19,23</sup>, and 3) chromosome C-banding technique<sup>7,13,14</sup>. Using the first method, a translocated chromosome is detected by its pairing with a marker chromosome in tester stocks. Wheat-rye addition lines, which contain an added pair of rye chromosomes in a wheat genome, are utilized for detecting wheat-rye translocations. Translocations are detected by the second method through the expression of rye genes for phenotypes and isozyme markers in wheat. The most recently developed method for identifying wheat-rye translocations is C-banding, which produces distinct heterochromatin banding patterns on the 21 chromosomes of wheat, and the 7 chromosomes of rye. While all three methods are useful for detecting wheat-rye translocations, only the second method is capable of detecting the incorporation of small pieces of rye chromosomes<sup>6</sup>. For plant breeding purposes, the in-

corporation of smaller pieces of alien DNA is more desirable. Hence, the development of more efficient and sensitive methods of detecting wheat-rye translocations is important.

Several studies have used the method of in situ hybridization to identify the chromosomes of wheat and rye<sup>1,10,11,16</sup>. The method, which involved the annealing of radioactive probes containing repeated DNA sequences of wheat or rye to cytological preparations, have been shown to produce specific hybridization patterns on the chromosomes. In rye chromosomes, the hybridization patterns observed are similar to C-banding patterns<sup>11</sup>. Recent work demonstrated the application of in situ hybridization to distinguish the chromosomes of wheat and rye in triticale<sup>1</sup>, and to identify a wheat-rye translocation<sup>16</sup>. Probes that recognized specific sites of highly repeated sequence DNA in either wheat or rye chromosomes were utilized.

Recently, the in situ hybridization technique has been modified with the use of biotin, instead of radioisotopes, to label the DNA probes<sup>12,18</sup>. Biotin-labeled probes have been shown to give a resolution that is comparable to that obtained with probes of high specific radioactivity<sup>12</sup>. In plants, this labeling technique was developed by Rayburn and Gill<sup>18</sup>. Their technique allowed the accurate determination of hybridization sites of a repeated sequence to wheat chromosomes, and

Drs. Lapitan and Sears are affiliated with the Department of Agronomy, and Drs. Rayburn and Gill with the Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, KS 66506. The authors appreciate the assistance of D. Wilson and W. J. Raupp in the processing of colored prints. Research supported by U.S. Department of Agriculture grant no. 59-2201-1-1-639-0. Contribution No. 86-233-J, Kansas Agricultural Experiment Station, Kansas State University. Please address reprint requests to Dr. Sears.  
© 1986, American Genetic Association.



**FIGURE 1** In situ hybridization of biotin-labeled pSC 119 probe to chromosomes of *A*—*S. cereale* cv. Chaupon; *B*—*T. aestivum* cv. Chinese Spring-Imperial rye addition line 4R; *C*—*T. aestivum* cv. Amigo, and *D*—*T. aestivum* cv. Bobwhite. The distinguishable hybridization patterns between the chromosomes of wheat (blue color) and rye (brown color) allow the rapid

detection of rye chromosomes and wheat-rye translocations in wheat-rye addition lines (*B*) and translocation lines (*C* and *D*), respectively. Arrows indicate the rye chromosomes in *B*, one of which is a telosome, and wheat-rye translocations in *C* and *D*.

required a much shorter time than conventional in situ hybridization.

In this study, in situ hybridization with biotin-labeled probes was utilized to detect wheat-rye translocations. A 120-bp repeated sequence from rye was used as a probe, be-

cause it exhibited distinguishable hybridization patterns to the chromosomes of wheat and rye. The hybridization patterns to wheat and rye chromosomes are described, and the technique for detecting translocations and chromosome breakpoints is discussed.

#### Materials and Methods

*Triticum aestivum* (AABBDD) cvs. ND7532, Amigo, Bobwhite, and Chinese Spring with Hairy-Neck 2 translocation (HN-2) were analyzed by in situ hybridiza-

tion. Amigo, Bobwhite, and HN-2 have been previously identified to contain wheat-rye translocations by the transfer of greenbug resistance<sup>9</sup>, mildew and yellow rust resistance<sup>17,23</sup> and hairy-neck trait<sup>19</sup>, respectively, from rye into wheat. The translocations involve the following wheat and rye chromosomes: 1A and 1R in Amigo, 1B and 1R in Bobwhite, and 5B and 5R in HN-2. *Secale cereale* cv. Chaupon and wheat-rye addition lines (Chinese Spring/Imperial 1R to 7R), also were used. Seed of these materials were germinated. Root tips were then collected according to Endo and Gill<sup>5</sup>, and used for squash preparations.

Clones pSC 119 and pSC 74, obtained from Dr. R. Flavell, Plant Breeding Institute, Cambridge, England, were used as probes. Highly repeated sequences from rye with repeating units of 120-bp and 480-bp were contained in clones pSC 119 and pSC 74, respectively<sup>3</sup>. The probes were labeled with biotin, and used for *in situ* hybridization. The details of the procedure for nick translation, *in situ* hybridization, and detection of hybridization sites were as described by Rayburn and Gill<sup>18</sup>.

Chromosomal regions that hybridized with the probe were identified by their brown color, which resulted from the enzymatic reaction between peroxidase and diaminobenzidine tetrahydrochloride (DAB) during the detection step. Chromosomal regions not hybridizing with the probes were distinguished from the hybridization sites by their blue color, which resulted from Giemsa staining.

The hybridization patterns of pSC 119 and pSC 74 to chromosomes of rye and wheat were compared to hybridization patterns reported for the rye cv. King II<sup>11</sup> and wheat cv. Chinese Spring<sup>18</sup>, respectively. Tritium-labeled probes were utilized in the previous study of King II and other rye cultivars<sup>11</sup>. pSC 119 probe was used to detect the wheat-rye translocations in Amigo, Bobwhite, and HN-2. The translocations were detected by the appearance of hybridization patterns characteristic for rye and wheat in the same chromosome.

C-banding was conducted on Amigo, Bobwhite, and HN-2 according to Lukaszewski and Gustafson<sup>14</sup>. The karyotypes were analyzed and compared with the standard karyotypes of rye cv. Chaupon and wheat cv. ND7532<sup>13</sup>. The wheat and rye chromosomes consisting the short arm (S) and long arm (L) of the translocations were identified.

Photographs were taken with a Zeiss III photomicroscope, using either Kodak Tech Pan 2415 or Ektachrome 50 film. Colored prints were made using Cibachrome II processing.

## Results

### *In situ* hybridization patterns in chromosomes of rye and wheat

The 480-bp repeated sequence hybridized to all the seven chromosomes of rye in Chaupon and the wheat-rye addition lines, mainly at the telomeres and at a few interstitial sites. The hybridization sites corresponded to the location of heterochromatic regions, and were similar to those detected previously by autoradiography in King II, and other rye cultivars<sup>11</sup>. The 480-bp repeat did not hybridize to any of the wheat chromosomes, since it is specific to rye<sup>3</sup>.

The 120-bp repeated sequence also hybridized to the seven rye chromosomes. But unlike the 480-bp repeat, the hybridization sites of the 120-bp repeat occurred along the entire length of each chromosome, resulting in the brown-colored appearance of all rye chromosomes (Figure 1A). Most of the sites at the telomeres and some interstitial regions appeared as distinct, dark brown bands, indicating strong hybridization of the probe. All of these sites, except the site on the nucleolus organizer region (NOR), were previously observed in King II<sup>11</sup>. However, this is the first time that the other hybridization sites, which were distributed all over the rye chromosomes, were detected. These sites appeared to be more weakly labeled, as shown by their lighter brown color. Because of the labeling of entire chromosomes of rye, individual chromosomes were difficult to distinguish by the hybridization patterns. Only chromosome 1R could be identified easily by the presence of the NOR site on its short arm.

The 120-bp repeated sequence was observed to hybridize to 11 of the 21 chromosomes of wheat in ND7532 and the wheat-rye addition lines, consistent with the results of Rayburn and Gill<sup>18</sup>. But the distribution of hybridization sites was very different from that observed in rye chromosomes. The hybridization sites were clustered at a few distinct sites, producing a predominant blue color in the wheat chromosomes. The contrast in the hybridization patterns between the chromosomes of wheat and rye were evident in the wheat-rye addition lines, where the rye chromosomes were distinguished readily by their brown color (Figure 1B).

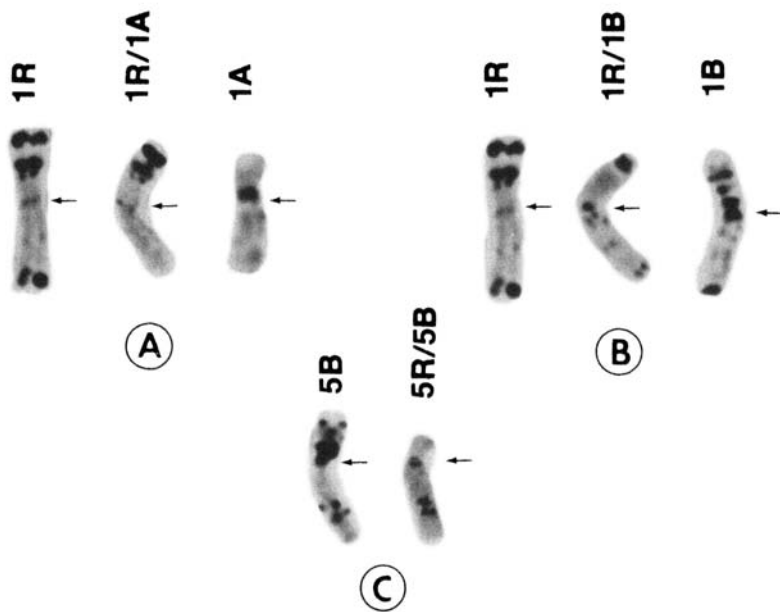
### Detection of wheat-rye translocations by *in situ* hybridization

Because of the characteristic distribution of the 120-bp repeat in the chromosomes of wheat and rye, it was tested for its usefulness in detecting wheat-rye translocations. *In situ*

hybridization using the biotin-labeled pSC 119 probe on the cultivars Amigo, Bobwhite, and HN-2 allowed the detection of wheat-rye translocations by their hybridization patterns (Figure 1C and D). All three chromosome translocations were characterized by the presence of one arm that was heavily labeled and appeared brown, and another arm that was labeled only at a few specific sites and appeared blue. Consequently, these chromosomes were easily recognized to consist of rye and wheat chromosomes. Furthermore, they were distinct from the other labeled wheat chromosomes.

The exact breakpoints in the three translocations were determined, based on the hybridization patterns. In Amigo, the breakpoint was at the centromere, with the short arm consisting of chromatin from rye chromosome 1R (Figure 1C). The NOR site was evident, indicating that it came from the short arm. The long arm was not labeled, and was easily recognized to contain a wheat chromosome. In Bobwhite, the translocation also involved a centromeric breakpoint (Figure 1D). The short arm consisted of rye chromatin from 1RS. In this chromosome, the long arm also was labeled, but it could still be distinguished to consist of a wheat chromosome. By the hybridization pattern, this chromosome arm was identified to have come from 1BL. The breakpoint in the HN-2 translocation was at the centromere. The short arm consisted of a very small chromosome segment that was labeled throughout, and was thus determined to consist of rye chromatin. The long arm contained a faint interstitial band in the middle, and was identified to be wheat chromosome 5BL.

The efficiency of this technique for detecting wheat-rye translocations was compared with C-banding, the usefulness of which already has been demonstrated<sup>7,13,14</sup>. The translocated chromosomes in Amigo and Bobwhite were identified by the appearance of large telomeric C-bands characteristic for rye chromosome 1RS (Figure 2A and B). The wheat chromosome involved in the translocations were identified to be 1AL and 1BL for Amigo and Bobwhite, respectively. The HN-2 translocation was recognized by the disappearance of the diagnostic band in the short arm of wheat chromosome 5B, and its replacement by a non-heterochromatic chromosomal region (Figure 2C). Without prior knowledge of the presence of a rye chromosome in this arm, however, its identification by C-banding alone was not possible. The breakpoints of the translocations were determined to be centromeric in Bobwhite and HN-2 (Figure 2B and C). In Amigo, the



**FIGURE 2** C-banding analysis of wheat-rye translocations. *A*—normal 1R, 1R-1A translocation in Amigo, normal 1A. *B*—normal 1R, 1R-1B translocation in Bobwhite, normal 1B. *C*—normal 5R, 5R-5B translocation in HN-2. Normal rye and wheat chromosomes are from Chaupon and ND7532, respectively. Arrows indicate breakpoints in chromosomes.

breakpoint also was centromeric, but was difficult to ascertain by banding alone.

### Discussion

The results showed the usefulness of *in situ* hybridization utilizing a biotin-labeled probe for detecting wheat-rye translocations. The probe used (pSC 119) hybridized to the entire length of all rye chromosomes, but only to a few specific sites in wheat chromosomes. As a result of the enzymatic detection procedure used in conjunction with biotin-labeling, the rye chromosomes appeared brown, whereas the wheat chromosomes appeared blue, except at a few hybridization sites. The distinguishable appearance between the chromosomes of wheat and rye resulted in a rapid and efficient means of detecting wheat-rye translocations.

The use of *in situ* hybridization had several advantages over C-banding for detecting wheat-rye translocations. First, C-banding was capable of recognizing translocated rye chromosome segments only if these contained heterochromatic C-bands. In contrast, *in situ* hybridization with the biotin-labeled pSC 119 probe recognized both heterochromatic and euchromatic regions of rye chromosomes. A case in point was the HN-2 translocation, where the presence of a small rye chromosome segment in the short arm could not be determined by C-banding alone

because of the absence of bands, but could be detected by *in situ* hybridization. Second, *in situ* hybridization allowed the accurate determination of the breakpoints in the translocations, and of the amount of rye chromatin that has been incorporated into wheat. With C-banding, the exact breakpoints could not be determined in the absence of diagnostic bands around the breakpoint. Therefore, the *in situ* hybridization technique was much more sensitive than C-banding.

The ability of the biotin-labeled pSC 119 probe to label all the chromosomal regions of rye indicates its usefulness in detecting the transfer of small rye chromosome segments, which may not be detected by other cytological methods. *In situ* hybridization using the biotin-labeled pSC 119 probe can thus accelerate the utilization of wheat-rye translocations in wheat breeding research, where the goal is to incorporate rye genes of economic importance, without the excess chromatin, which may produce detrimental effects on wheat.

The difference between the observed hybridization patterns of the 120-bp repeat with that previously observed by Jones and Flavell<sup>11</sup> may be attributed to the method of labeling employed. The weakly labeling sites occurring throughout the rye chromosomes, may represent regions containing low copy numbers of the 120-bp repeat<sup>2</sup>, explaining the failure to be detected with tritium label-

ing and autoradiography. On the other hand, the ability to detect these sites with biotin may reflect the increased resolution obtained with this labeling method. Alternatively, the differences may have been due to the difference in hybridization conditions used. The absence of the NOR site in the previous study may be due to intervarietal differences.

We have demonstrated a method of detecting wheat-rye translocations and chromosome breakpoints with increased efficiency and sensitivity, using a biotin-labeled rye DNA probe which is capable of labeling both heterochromatic and euchromatic regions of rye chromosomes. The cloning of other dispersed repeats that are specific for the genomes of other related species will provide useful cytogenetic tools for detecting the introgression of alien DNA into wheat.

### References

- APPELS, R., J. P. GUSTAFSON, and C. E. MAY. Structural variation in the heterochromatin of rye chromosomes in triticales. *TAG* 63:235-244. 1982.
- BEDBROOK, J., J. JONES, and R. FLAVELL. Evidence for the involvement of recombination and amplification events in the evolution of *Secale* chromosomes. *Cold Spring Harbor Symp. Quant. Biol.* 45:755-760. 1981.
- \_\_\_\_\_, M. O'DELL, R. D. THOMPSON, and R. B. FLAVELL. A molecular description of telomeric heterochromatin in *Secale* species. *Cell* 19:545-560. 1980.
- DRISCOLL, J. C. and N. F. JENSEN. Characteristics of leaf rust resistance transferred from rye to wheat. *Crop Sci.* 4:372-374. 1964.
- ENDO, T. R. and B. S. GILL. Somatic karyotype, heterochromatin distribution, and nature of chromosome differentiation in common wheat, *Triticum aestivum* L. em Thell. *Chromosoma* 89:361-369. 1984.
- FLAVELL, R. B., M. O'DELL, and J. JONES. Cereal genome studies and plant breeding research. In *Genetic Engineering of Crops. I.* Rubenstein, P. L. Phillips, and C. E. Green, Eds., University of Minnesota Press, Minneapolis. p. 76-90. 1980.
- GILL, B. S. and G. KIMBER. Recognition of translocations and alien chromosome transfers in wheat by the Giemsa C-banding technique. *Crop Sci.* 17:264-266. 1977.
- HART, G. E., D. E. McMILLIN, and E. R. SEARS. Determination of the chromosomal location of a glutamate oxaloacetate transaminase structural gene using *Triticum agropyron* translocation. *Genetics* 83:49-61. 1976.
- HOLLENHORST, M. M. and L. R. JOPPA. Chromosomal location of genes for resistance to greenbug in 'Largo' and 'Amigo' wheats. *Crop Sci.* 23:94-96. 1983.
- HUTCHINSON, J., R. B. FLAVELL, and J. JONES. Physical mapping of plant chromosomes by *in situ* hybridization. In *Genetic Engineering*, Vol. 3. J. K. Setlow and A. Hollaender, Eds., Plenum Publishing, NY. p. 207-222. 1981.
- JONES, J. D. G. and R. B. FLAVELL. The mapping of highly repeated DNA families and their relationship to C-bands in chromosomes of *Secale cereale*. *Chromosoma* 86:595-612. 1982.
- LANGER-SAFER, P. R., M. LEVINE, and D. C. WARD. Immunological method for mapping genes on *Drosophila* polytene chromosomes. *PNAS* 79:4381-4385. 1982.

13. LAPITAN, N. L. V., R. G. SEARS, and B. S. GILL. Translocations and other karyotypic structural changes in wheat X rye hybrids regenerated from tissue culture. *TAG* 68:547-554. 1984.
14. LUKASZEWSKI, A. J. and J. P. GUSTAFSON. Translocations and modifications of chromosomes in triticale X wheat hybrids. *TAG* 64:239-248. 1983.
15. MARTIN, T. J., T. L. HARVEY, and R. W. LIVERS. Resistance to wheat streak mosaic virus and its vector, *Aceria tulipae*. *Phytopathology* 66:346-349. 1976.
16. MAY, C. E. and R. APPELS. Rye chromosome translocations in hexaploid wheat: a re-evaluation of the loss of heterochromatin from rye chromosomes. *TAG* 56:17-23. 1980.
17. METTIN, D., W. D. BLUTHNER, and G. SCHLEGEL. Additional evidence on spontaneous 1B/1R wheat-rye substitutions and translocations. Proc. 4th Int. Wheat Genet. Symp., Missouri Agricultural Experiment Station, Columbia, MO. p. 179-184. 1973.
18. RAYBURN, A. L. and B. S. GILL. Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. *J. Hered.* 76:78-81. 1985.
19. SEARS, E. R. Induced transfer of hairy neck from rye to wheat. *Z. Pflanzenzucht.* 57:4-25. 1967.
20. SEBESTA, E. E. and E. A. WOOD, JR. Transfer of greenbug resistance from rye to wheat with x-rays. *Agron. Abst.* 1978:61-62. 1978.
21. SHARMA, H. C. and B. S. GILL. Current status of wide hybridization in wheat. *Euphytica* 32:17-31. 1983.
22. STEWART, D. M., E. C. GILMORE, JR. and E. R. AUSEMUS. Resistance to *Puccinia graminis* derived from *Secale cereale* incorporated into *Triticum aestivum*. *Phytopathology* 58:508-511. 1968.
23. ZELLER, F. J. 1B/1R wheat-rye chromosome substitutions and translocations. Proc. 4th Int. Wheat Genet. Symp., Missouri Agric. Exper. Station, Columbia, MO. p. 209-211. 1973.